

Symposium: Dietary Zinc and Iron—Recent Perspectives Regarding Growth and Cognitive Development

Behavioral Data and Methodology Issues in Studies of Zinc Nutrition in Humans^{1–4}

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ABSTRACT Despite the widespread incidence of childhood zinc (Zn) deficiency and strong evidence that Zn deprivation during periods of rapid growth affect brain development and behavior in animals, there is little research on the behavioral effects of Zn deficiency in children or adults. A brief review of previous human studies is followed by more detailed discussion of recent studies of Chinese and Mexican-American children, which showed beneficial effects of Zn repletion on neuropsychologic function. Methodology issues are reviewed and recommendations are made to assess the following: 1) a broad range of cognitive, psychomotor, emotional and social factors; 2) performance in the presence of secondary stressors to approximate real-world conditions more accurately; 3) continuous activity and rest in older children by the use of electronic activity monitors; and 4) electrophysiologic measures of brain function. It is concluded that research on cognition, behavioral activity and brain electrophysiology as outcomes of Zn deficiency and response to improved Zn nutrition is critical, given that Zn deficiency is common in both developing and developed countries. *J. Nutr.* 130: 361S–364S, 2000.

KEY WORDS: • zinc nutrition • humans • cognition • brain function • methods

Zinc (Zn) deficiency in children continues to be a modern nutritional and health problem in both developing (Gibson 1994) and developed (Sandstead 1995) countries. Despite substantial evidence from animal experiments showing that Zn deprivation during periods of rapid development impairs behavior and brain development in animals (Golub et al. 1995, Halas and Eberhardt 1987), few studies have investigated Zn deficiency effects on behavior or brain function of children.

Zn and behavior in infants

Several studies have examined the relationship between Zn nutrition and motor development and activity in infants. Friel et al. (1993) supplemented very-low-birth-weight U.S. infants with either 6.7 or 11 mg Zn/L from birth to 5 mo and found

better motor development on the Griffiths developmental assessment in the infants receiving higher Zn. In a study of Egyptian infants, a relationship was found between mothers' consumption of foods high in available Zn and attention measured by the Brazelton Neonatal Development Assessment Scales administered shortly after birth (Kirksey et al. 1991), and with motor development measured by the Bayley Scales of Infant Development administered at 6 mo (Kirksey et al. 1994). Sazawal et al. (1996) found increased activity, particularly among boys, after 6 mo of repletion with 10 mg Zn/d added to a multivitamin mixture in Indian infants and toddlers initially aged 6–17 mo, compared with children given only multivitamins. Similarly, in 6- to 9-mo-old Guatemalan infants, Bentley et al. (1997) observed increased activity after 7 mo of supplementation with 10 mg Zn/d, compared with control infants. In a recent study of Brazilian infants (aged 6–12 mo), repletion with 5 mg Zn/d increased responsiveness and decreased problem behaviors associated with low birth weight, but had no effect on mental or psychomotor function measured by the Bayley Scales of Infant Development (Ashworth et al. 1998).

Zn and behavior in school-aged children

In older children, Thatcher et al. (1984) reported a positive correlation between hair Zn and reading ability in elementary school children living in the Baltimore, MD area. However, Zn repletion (10 mg/d) studies with Canadian boys (aged 5–7 y) and Guatemalan boys (aged 6–7 y) failed to find changes in performance on a subset of tests from the Detroit Test of Learning

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Abilities (Cavan et al. 1993, Gibson et al. 1989). More recently, Zn intakes of Egyptian children (aged 7–10 y) were found to correlate with social behavior of girls and activity level of boys, but not with cognitive function (Wachs et al. 1995).

Penland et al. (1997 and 1998) and Sandstead et al. (1998) used a more extensive neuropsychologic test battery (Cognition Psychomotor Assessment System-Revised; Penland 1994) to measure cognitive and psychomotor function of 740 urban and 540 rural Chinese children (aged 6–9 y) in response to repletion with 20 mg Zn/d (Zn), a micronutrient mixture (M), or a combination of the two (ZnM). Findings in the urban phase of this project were as follows. Compared with M, ZnM resulted in greater improvement in recognition memory (delayed design matching), reasoning (oddity) and psychomotor function (tapping and tracking). Zn also resulted in greater improvement than M in attention (continuous performance), perception (design matching), reasoning (oddity) and psychomotor function (tapping). Findings in the rural phase were as follows. Compared with M, ZnM resulted in greater improvement in attention (object pair search), recognition memory (delayed object search) and reasoning (oddity). Zn also resulted in greater improvement than M in recognition memory and reasoning. There were no treatment effects on performance of tasks emphasizing perceptual processes. The consistent finding among both urban and rural children was that Zn supplementation resulted in greater improvement than control treatments in performance on tasks assessing visual recognition memory and reasoning.

This research with Chinese children was the first to find a relationship between Zn intake and cognition and psychomotor function in older children. The failure of previous intervention studies by Gibson (Cavan et al. 1993, Gibson et al. 1989) to show this relationship may be explained by differences in intervention and performance assessment. The particulars of Penland's studies (Penland et al. 1997 and 1998) are as follows: 1) the Zn treatments provided twice the Zn used in Gibson's studies (20 vs. 10 mg/d); 2) treatment with Zn alone was compared with treatment with a multiple micronutrient supplement and with Zn treatment that was simultaneous with micronutrients to control for possible effects of other limiting nutrients (Ronaghy et al. 1974); 3) CPAS-R (Penland 1994) was configured to assess all neurocognitive domains, except language processing, deemed necessary for a comprehensive neuropsychologic evaluation of school-aged children (Taylor and Fletcher 1990); thus, a larger number of cognitive domains were assessed than in the Gibson study; and 4) cognitive measures assessed both speed and accuracy to detect performance trade-offs made to compensate for nutritional stressors (Wickelgren 1977). These differences exemplify several methodological issues that must be considered when designing studies of the behavioral and cognitive sequelae of Zn nutrition.

Penland et al. (1999) recently measured the beneficial effects of short-term Zn repletion of 240 low income Mexican-American children (aged 6–9 y) at risk for Zn deficiency because their usual diets were high in phytate. Because iron deficiency commonly coexists with Zn deficiency (Allen 1998, Darnell and Sandstead 1991), the effects of iron supplementation were also examined. Treatments were a micronutrient mixture (M; Sandstead et al. 1998), 20 mg Zn/d plus micronutrients (ZnM), 24 mg Fe/d iron plus micronutrients (FeM), or a placebo (P) administered 5 d each week for 10 wk in a double-blind control trial. CPAS-R (Penland 1994) was again configured to assess all cognitive domains except language processing. Examiners received both training and practice in administering the battery, and were instructed on when and

how to motivate effectively and reassure children to perform at their best. Testing was done in rooms free of visual as well as auditory distraction. Children were given thorough instructions and a practice session before data collection. The Child Behavior Checklist and Teacher Report Form (Achenback and Edelbrock 1986a and 1986b) were administered to assess psychoeducational performance, behavioral problems and social competencies.

Compared with the other treatments and placebo, ZnM resulted in significantly ($P < 0.05$) greater improvements in reasoning, indicated by the fact that fewer trials were needed to learn simple concepts ($M \pm \text{SEM}$: $M = -14 \pm 5$; $\text{ZnM} = -28 \pm 5$; $\text{FeM} = -12 \pm 4$, $P = -12 \pm 4$). Although significant effects of treatment with Zn were few, finding a beneficial effect of Zn supplementation on reasoning is noteworthy because it represents the third time this effect has been observed.

Zn and behavior in adults

There have been four experimental studies of Zn intakes or status and behavior in adult humans. Henkin et al. (1975) reported increased emotional lability and perceptual and memory deficits in patients with progressive systemic sclerosis after administration of histidine, a Zn chelating agent. Tucker and Sandstead (1984) found that men with low Zn intakes (<4 mg/d) had faster, but less accurate performance on a memory for digits task and several perceptual tasks. Darnell and Sandstead (1991) found that 30 mg Zn/d added to a vitamin-mineral supplement improved visual memory in sideropenic women, whereas the vitamin-mineral supplement alone did not. In a highly controlled 6-mo, live-in metabolic study, Penland (1991) found poorer performance on 9 of 15 cognitive and psychomotor tasks administered to men fed 1, 2, 3 or 4 mg Zn/d during each of four consecutive 35-d Zn deprivation periods assigned in a random, double-blind manner, compared with when they were fed a control diet containing 10 mg Zn/d.

Zn and brain electrophysiology in humans

There are some limited data showing a relationship between Zn intakes or status and brain electrophysiology. Several studies examined this relationship in clinical populations. For example, Tang (1991) reported low hair Zn concentrations in 55 women with epilepsy. Using a more controlled approach, Goldstein and Pfeiffer (1978) administered Zn or a placebo to schizophrenics and recorded their electroencephalogram (EEG) 4 h after treatment; they found a greater reduction in EEG amplitude (toward normal) in treated subjects compared with controls. Findings from these studies suggest that low Zn is associated with cortical hyperexcitability; however, the reports were incomplete and did not specify the specific EEG frequencies affected. At least three studies have investigated Zn effects on brain electrophysiology of healthy individuals. Henrotte et al. (1977) found that healthy subjects who showed decreased frequency responses in the EEG during hyperventilation also had lower red blood cell Zn concentrations than controls. Sandstead et al. (1983) found a correlation between plasma Zn and EEG activity in three of five subjects participating in a metabolic study of Zn depletion. The principal finding was increased left and decreased right hemisphere amplitudes in the occipital lobes in the lower frequencies of the EEG. Thatcher et al. (1984) reported that hair Zn concentrations of elementary school children

were correlated with EEG coherence involving the frontal lobe. That study also found that both hair Zn and EEG coherence were related to reading ability. Studies with healthy individuals seem to suggest that low Zn intakes or status are associated with highly specific effects involving specific areas of the brain and specific EEG frequencies and characteristics.

Methodology issues relevant to research on Zn and behavior

Methodology issues relevant to the study of behavioral consequences of Zn deficiency in children are similar to those in studies of behavior and nonspecific malnutrition. A workshop held by the International Dietary Energy Consultative Group (IDECG) in 1993 (IDECG 1995) produced several excellent reviews of these issues (e.g., Grantham-McGregor 1995); more recent discussions can be found in Allen (1998) and Black (1998). Several of these issues were addressed in the review noted above. Some specific points and suggestions for future research follow.

Individual differences, sex and age are extremely important concerns when designing research and analyzing results to assess relationships between Zn nutriture and behavior and brain function (Gorman 1995). Effects may be detected only by designs that yield relative response measures (i.e., changes in scores) and statistical analyses that differentiate outcomes by sex and age (Black 1998). Zn deficiency research involving children is implemented in the context of ongoing developmental changes, and the selection of interventions, outcome measures and statistical analyses must always reflect this fact.

As appears to be the case with general malnutrition, effects on motor development, activity level, exploratory activities and play, social skills and emotional states may mediate observed effects of Zn deficiency or supplementation on cognition and school achievement. Therefore, these potential mediating variables must be assessed. Further, ability to deal effectively with stressors may be another active intervening variable requiring assessment. Stress responses are highly compensatory in nature, acting to conserve the integrity of responses (e.g., performance) by drawing on other available resources. Because Zn deficiency itself is a stressor, its effect on neuropsychologic functions may be missed unless evaluated by means of a challenge test. Such tests are easy to contrive and may consist simply in the addition of a secondary task or stressor (e.g., noise) to the test situation. Properly devised, a challenge test may reveal whether the inability to compensate for the challenge is due to Zn deficiency or some other factor such as fatigue, impaired emotionality or poor problem-solving skills.

Do we seek consistent changes on a specific measure or consistent changes in a class of measures assessing a meaningful underlying function? The answer must consider the powerful compensatory mechanisms that are always available to reallocate resources to produce and preserve a desired outcome. Further, there must be a balance between the need to use highly focused tasks to assess specific neuropsychologic processes and the need to ensure that experimental tasks are sufficiently valid ecologically that they yield findings that generalize to the real world. Rather than seek consistency on a single experimental and sometimes arbitrary measure, I suggest we seek a convergence of evidence from varying approaches, interventions, populations and outcomes to discover the true picture of how Zn nutriture relates to behavior.

Given findings that infant and toddler activity may be impaired by Zn deficiency, objective assessment of activity should be considered for inclusion in studies of older children. Monitoring activity via electronic device is simple and relatively inexpensive, and permits determination of sleep patterns as well as gross motor activity during waking. Inadequate amounts of sleep and poor sleep quality can lead to fatigue, decreased performance efficiency and impaired social interactions. Last, addition of electrophysiologic measures of brain function as outcomes is proposed to expand assessment of functional sequelae of Zn deficiency and perhaps provide insight into the mechanisms responsible for Zn effects on behavior.

SUMMARY

Research on the beneficial effects of improved Zn nutrition for behavior and brain function is critically needed because Zn deficiency continues to be a modern health concern in both developing and developed countries. However, addressing the many methodologic issues inherent in this research presents a formidable challenge.

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